Chronic stress parameters in pigs

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Chapter 4

Effects of Rearing Conditions on Behavioural and Physiological Responses of Pigs to Preslaughter Handling and Mixing at Transport

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ABSTRACT

The physiological and behavioural responses of slaughter pigs reared in either a barren environment or in an enriched environment (larger pens with strawbedding) to preslaughter handling and mixing at transport were studied. Enriched reared pigs had higher salivary cortisol concentrations in the home pen, before and after transport than barren housed pigs, but at the end of the lairage period salivary cortisol concentrations did not differ between enriched and barren housed pigs. In pigs reared in a barren environment, salivary cortisol concentrations increased significantly after transport and being in lairage as compared to home pen salivary cortisol concentrations. In pigs reared in an enriched environment salivary cortisol concentrations did not significantly change after any stage of preslaughter handling and transport as compared to home pen salivary cortisol concentrations. In the lorry, pigs reared in a barren environment tended to spend more time walking, standing and manipulating other pigs than enriched reared pigs. Moreover, pigs reared in a barren environment tended to spend more time walking and fighting in lairage than enriched reared pigs. Results showed that rearing conditions may affect the behavioural and physiological responses of pigs to preslaughter handling and mixing at transport. The tendency for increased manipulation of pen mates and fighting, and the significant increases in salivary cortisol in response to mixing at transport and being in lairage in pigs reared in a barren environment, indicate that pigs reared in a barren environment are likely to experience more stress during common preslaughter procedures than pigs reared in an enriched environment.

Chapter 4

INTRODUCTION

Rearing conditions affect the behaviour and physiology of pigs during rearing (Beattie et al., 1995a, 1996; De Jong et al., 1998; De Jonge et al. 1996; Schouten, 1986). Pigs reared in a barren environment showed more manipulative social behaviour like biting, nosing and massaging of pen mates (Beattie et al., 1995a; De Jong et al., 1998; Schouten, 1986) and behaved more aggressively (De Jonge et al., 1996; O'Connell and Beattie, 1999) than pigs reared under enriched conditions, i.e. in larger pens with strawbedding. Moreover, pigs reared in a barren environment had lower baseline salivary cortisol concentrations during the light period (De Jong et al., 1998) as compared to pigs reared under enriched conditions. We showed that the lower baseline salivary cortisol concentrations in the light period in barren housed pigs were due to a blunted circadian rhythm in cortisol in barren housed pigs as compared to enriched housed pigs. Enriched housed pigs had higher salivary cortisol concentrations during the light period than barren housed pigs, whereas during the dark period enriched and barren housed pigs did not differ in baseline salivary cortisol concentrations (De Jong et al., 2000).

During preslaughter treatment and transport to the slaughterhouse, pigs are confronted with many stimuli eliciting stress responses, e.g. loading and unloading on the lorry, confrontation with novel environments, mixing with unfamiliar conspecifics, unusual sounds and motion of the lorry (Bradshaw et al., 1996; Grandin, 1997; Geverink et al., 1998; Warriss 1998). Because pigs reared in a barren environment showed a higher behavioural reactivity to novel stimuli (Beattie et al., 1995b; De Jong et al., 1998; Stolba and Wood-Gush, 1980; Olsson et al., 1999), performed more abnormal aggressive behaviour and showed a higher level of aggression (De Jonge et al., 1996; O'Connell and Beattie, 1999; Schouten, 1986) than enriched reared pigs, it may be expected that rearing conditions affect the responses of pigs to preslaughter procedures and mixing at transport to the slaughterhouse.

Previous studies indicated that rearing conditions indeed affect the responses of pigs to preslaughter procedures. Pigs reared under barren conditions were easier to load, but had higher cortisol responses to transport than enriched reared pigs (Geverink et al., 1999). However, pigs in that study were not mixed with unfamiliar conspecifics in the lorry, whereas mixing is a common procedure when pigs are transported to the slaughterhouse (Bradshaw et al., 1996; Geverink et al.,

1996). Warriss et al. (1983) observed calmer behaviour of extensively reared pigs during loading but did not observe the behaviour during transport.

In the present experiment pigs reared in an enriched and barren environment were mixed at transport, and behavioural and physiological responses to preslaughter handling, transport and being in lairage were measured to gain insight in the effect of rearing conditions on the abilities to cope with common stressors in pig husbandry.

MATERIALS AND METHODS

All procedures were approved by the ID-Lelystad Animal Care and Use Committee (Lelystad, The Netherlands).

Animals and Housing

Fourty-eight crossbred slaughter pigs (Great Yorkshire x (Great Yorkshire x Dutch Landrace)) were used in this experiment. Two treatments, enriched (E) vs. barren (B) housing conditions, were applied to two successive replicates of 24 pigs. Within each replicate, three groups of four pigs were assigned to each treatment. Each group consisted of two barrows and two gilts from the same litter.

E piglets were born in farrowing pens where the sows were loose housed (7.2 m^2), with a concrete lying area covered with straw (1.75 x 2.4 m) and a concrete slatted area (1.25 x 2.4 m). B piglets were born in standard farrowing pens without substrate where the sows were crated (3.1 m^2, half concrete area, half metal slats). Piglets were weaned at 28 days of age and six piglets per sow (three barrows, three gilts) were randomly selected for use in this experiment. Piglets stayed in the same pen at weaning and the sow and not-selected piglets were removed. At 10 weeks of age a final selection of four experimental pigs per sow (two barrows, two gilts) was done. E pigs were relocated to enriched fattening pens (4.64 m^2) with half concrete area covered with straw and half concrete slats. B pigs were relocated to barren fattening pens (3.36 m^2) with half concrete lying area and half concrete slatted floor. E and B fattening pens were in the same room. Water and food were available *ad libitum*. Pens were cleaned daily and fresh straw was provided in the E pens at 08.30 h. Environmental temperature was kept between 19 to 21°C in each room. Artificial lights were on from 06.00 - 18.00 h.

Transport and Lairage Procedure

Pigs were slaughtered at an age of 25 weeks (mean weight E pigs: 109.9 ± 2.5 kg, B pigs 109.6 ± 2.6 kg) at the slaughterhouse of the Institute for Animal Science and Health (Lelystad, The Netherlands). For each replicate, the same procedure before slaughter was applied. Food was withdrawn approximately 20 h before slaughter. Pen-groups were separately loaded on the lorry. At 09.00 h, the first group of pigs was allowed to voluntarily leave their pen and gently driven to a transport box at the end of the passageway. The pigs were transported to the lorry, allowed to voluntarily leave the transport box and loaded. Pigs were mixed within a treatment. The E pigs were penned separately from the B pigs on the same deck of the lorry (0.46 m^2/pig); the lorry had one deck and only the experimental pigs were on the lorry. Each pen on the lorry thus contained 12 pigs. For the second replicate, the sequence of E and B pigs in the lorry was alternated. Transport to the slaughterhouse took 1 h. After arrival at the slaughterhouse, the E and B treatment were separately unloaded and penned separately in lairage. At 13.00 h, after a fixed lairage period of 105 min, pigs were driven one by one to the stunning pen and the pigs were manually stunned. E and B pigs were stunned alternately.

Saliva Collection and Cortisol Analysis

Saliva was collected from all pigs in the home pen after the provision of fresh straw for E pigs, at 08.30 h (t=0), immediately after being driven into the lorry and mixing (t=60 min, 09.30 h), after transport (t=160 min, 11.00 h), after being driven in lairage (t=180 min, 11.15 h), and at the end of the lairage period (t=270 min, 13.00 h). All saliva samples were taken during the circadian peak of the cortisol rhythm, i.e. between 8.30 - 13.00 h (De Jong et al., 2000). Saliva was collected by allowing the pigs to chew on two large cotton buds until they were thoroughly moistened (about 30-60 sec per sample). The buds were placed in tubes and centrifuged 10 min at 400 g. Saliva samples were stored at -20°C until analysis. Cortisol concentration in saliva samples was determined using a solid-phase radioimmunoassay kit (Coat-a-Count Cortisol TKCO, Diagnostic Products Corporation, Apeldoorn, The Netherlands) modified for pig salivary cortisol (Ruis et al., 1997). Cortisol in saliva is essentially in the free biologically active form, and a good indication of levels of cortisol in blood plasma (Parrott et al., 1989).

Behaviour

Behavioural parameters sampled at the experimental farm were (1) latency to leave the pen and (2) latency to leave the transport box to enter the lorry.

Inside the lorry, two cameras were mounted at the ceiling, each camera encompassing one pen. Continuous recordings were made during transport. In lairage, behaviour was continuously recorded with a camcorder until the first pig was stunned. Duration of behavioural elements were scored for each pig using the Observer software (Noldus, Wageningen, The Netherlands) for the following periods: (1) 10 min before the start of transport in the lorry, after mixing of the pigs, (2) first, (3) second, (4) third, (5) fourth 15 min of transport, (6) 10 min after transport in the lorry, (9) first, (10) second, (11) third, (12) fourth 15 min of the lairage period. Duration of the following behavioural elements was scored: (1) standing; (2) sitting; (3) lying; (4) walking; (5) exploration, i.e. sniffing, nosing or chewing the floor or walls of the pen; (6) manipulation of pigs, i.e. massaging, nosing and chewing of other pigs, (7) fighting, i.e. attacking with reaction of the opponent or opponents and biting involved; (8) other.

Statistical Analysis

Data were checked for normal distribution and homogeneity of variances. Differences in cortisol concentration and behaviour were analysed with a mixed analysis of variance model with treatment, replicate and sex as fixed effects in the model and litter (rearing group) entered as random effect. Behavioural data from the 3rd and 4th 15-min period of transport of the E pigs in the first replicate were omitted due to a failure in the recording equipment. Group means of latency to leave the pen and latency to leave the transport box were analysed with a mixed analysis of variance model with replicate as fixed effect and litter (rearing group) as random effect. Components were estimated with the Restricted Maximum Likelihood Model (REML) procedure (Genstat, 1993). Increase in salivary cortisol concentrations as compared to home-pen values were compared with a paired t-test for E and B pigs respectively.

RESULTS

Salivary Cortisol

E pigs had significantly higher salivary cortisol concentrations than B pigs in the home pen (8.2 ± 3.8 ng/ml vs. 3.0 ± 0.7 ng/ml for E vs. B pigs; $p<0.01$), after driving into the lorry and mixing (8.61 ± 1.88 ng/ml vs. 3.29 ± 0.73 ng/ml for E vs. B pigs; $p<0.05$), after transport (10.56 ± 0.95 ng/ml vs. 7.06 ± 0.38 ng/ml for E vs. B pigs; $p<0.05$) and at the beginning of the lairage period (9.59 ± 1.12 ng/ml vs. 4.30 ± 6.28 ng/ml for E vs. B pigs; $p<0.01$). E and B pigs did not differ significantly in salivary cortisol concentrations at the end of the lairage period (9.37 ± 1.74 ng/ml vs. 9.43 ± 1.92 ng/ml for E vs. B pigs).

Figure 1 shows the change in salivary cortisol concentration after preslaughter handling and transport as compared to the home pen concentrations. For E pigs, salivary cortisol concentrations after driving, after transport, at the beginning and at the end of the lairage period did not differ significantly from salivary concentrations measured in the home pen. For B pigs, salivary cortisol concentrations were significantly increased after transport ($p<0.01$) and at the end of the lairage period ($p<0.05$) as compared to the salivary cortisol concentrations measured in the home pen.

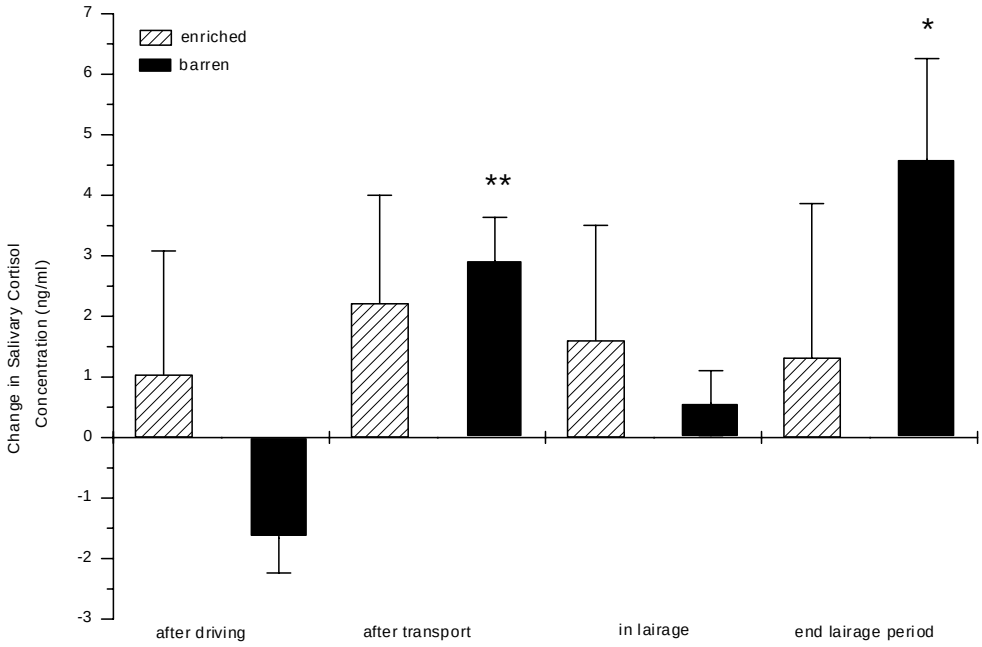

Figure 1. Changes in salivary cortisol concentration (mean ± s.e.m.) as compared to the initial home pen concentration after driving, mixing, transport and being in lairage for pigs reared in an enriched or in a barren environment. *p<0.05; **p<0.01 as compared to home pen concentrations for barren housed pigs.

Behaviour

E and B pigs did not differ significantly in latency to leave the home pen (24.6 ± 4.8 sec vs. 17.8 ± 2.4 sec for E vs. B pigs) and latency to leave the transport box to enter the lorry (17.9 ± 2.0 sec vs. 15.7 ± 2.5 sec for E vs. B pigs).

In the lorry, B pigs tended to spend more time walking, standing and manipulating other pigs than E pigs (Fig. 2). Differences between E and B pigs were found for the following time points: walking: before transport $p<0.05$, 4th 15 min of transport $p<0.01$; standing: 1st, 4th 15 min of transport and after transport $p<0.10$ (tendency); manipulating other pigs: before transport, 2nd 15 min and 4th 15 min of transport $p<0.10$ (tendency), after transport $p<0.05$. E pigs tended to spend more time lying than B pigs during transport (Fig. 2). This difference was significant at the 3rd 15 min of transport ($p<0.05$) and the 4th 15 min of transport ($p<0.01$). Almost no fighting was observed during transport (Fig. 2). E and B pigs did not differ in time spent sitting, exploring the pen and other behaviours (data not shown).

In lairage, B pigs tended to spend more time walking than E pigs (Fig. 3). Differences between E and B pigs were found for the 1st 15 min in lairage ($p<0.10$, tendency) and the 3rd 15 min in lairage, ($p<0.05$). Pigs of the first replicate walked significantly more in lairage than pigs of the second replicate ($p<0.01$ for all time points; data not shown). B pigs also tended to spend more time fighting than E pigs (Fig. 3). This difference was significant at the 3rd 15 min in lairage ($p<0.05$). Figure 3 shows that the time spent lying increased with time spent in lairage.

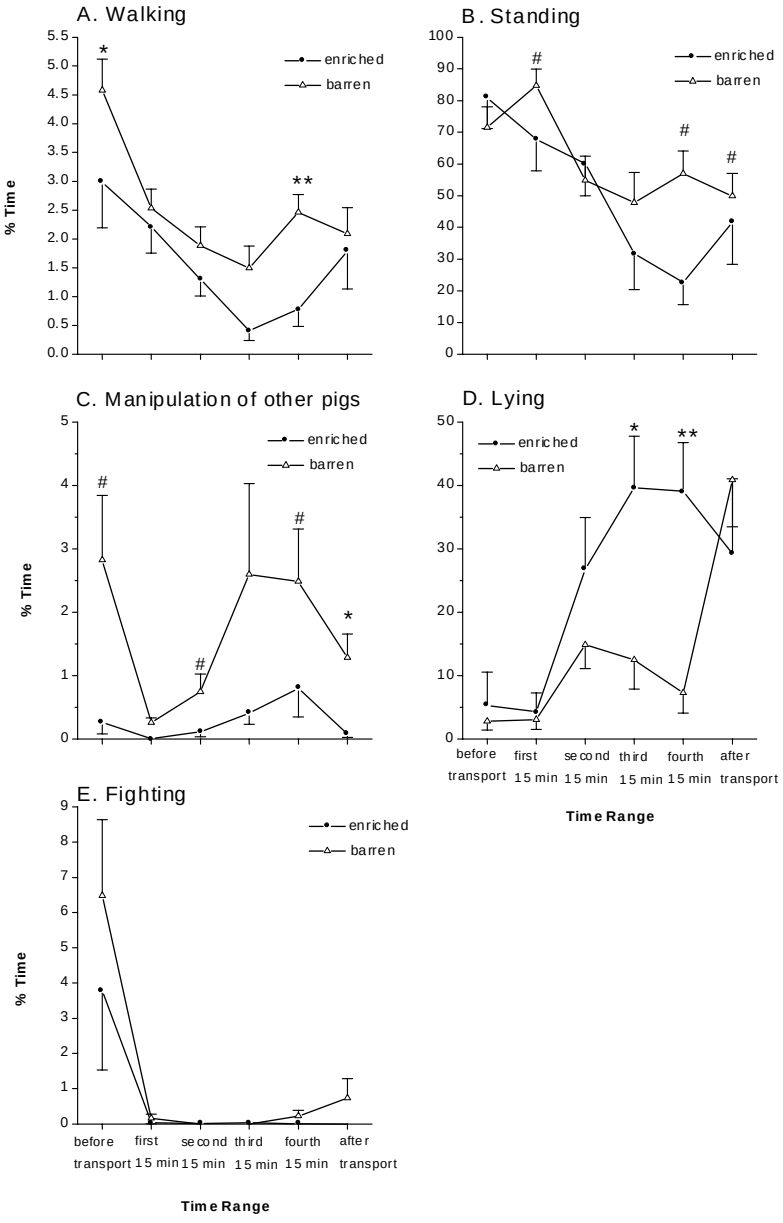

Figure 2. Behaviour in the lorry (mean ± s.e.m.) after mixing of pigs reared in an enriched or in a barren environment. $^{\#}p<0.10$ (tendency); *$p<0.05$; **$p<0.01$.

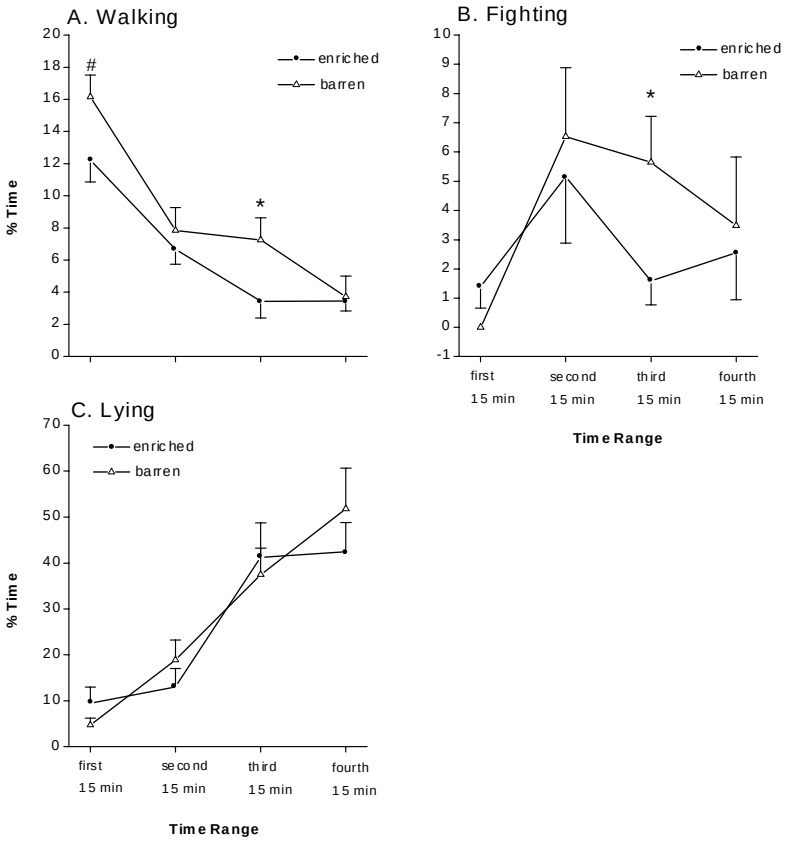

Figure 3. Behaviour in lairage (mean ± s.e.m.) of pigs reared in an enriched or in a barren environment. $^{\#}p<0.10$ (tendency); $^{*}p<0.05$.

DISCUSSION

This experiment showed that rearing conditions affect physiological and behavioural responses of pigs to preslaughter handling and mixing at transport. In B pigs, salivary cortisol concentrations increased significantly after transport and at the end of the lairage period as compared to salivary cortisol concentrations in the home pen. In E pigs, salivary cortisol concentrations after preslaughter handling and transport did not differ significantly from salivary cortisol concentrations in the home pen. In the lorry, pigs reared in a barren environment tended to be more active and to spend more time massaging, nosing or chewing other pigs than enriched reared pigs. In lairage, barren housed pigs tended to spend more time walking and fighting than enriched reared pigs.

Previous experiments showed that B pigs had lower baseline salivary cortisol concentrations during the light period as compared to E pigs, due to a blunted circadian rhythm in salivary cortisol in B pigs (De Jong et al., 1998; De Jong et al., 2000). The present data are consistent with the previous experiments, as B pigs had lower salivary cortisol concentrations in the home pen than E pigs. However, these levels were about twice as high for both E and B pigs as compared to baseline levels measured at 22 weeks of age in the same pigs (De Jong et al., 2000). This may be due to food deprivation stress, because food was withdrawn 20 h before transport to prevent travel sickness. It has been shown that after food deprivation salivary cortisol concentrations in pigs are increased (Parrott et al., 1989). However, it may also be possible that increased activity due to the provision of straw just before saliva sampling, or a dietary effect caused the high baseline salivary cortisol concentrations in E pigs. E pigs had the possibility to eat straw after food deprivation, in contrast to B pigs. It is unknown to what extent dietary fibre may affect salivary cortisol concentrations.

Salivary cortisol concentrations in B pigs increased significantly after transport and in lairage, in contrast to salivary cortisol concentrations in E pigs that did not increase significantly as compared to home pen salivary cortisol concentrations. This indicates that the Hypothalamo-Pituitary-Adrenal system may be more reactive in B pigs than in E pigs. Our results confirm a previous experiment, showing a higher increase in salivary cortisol in response to transport in barren housed pigs as compared to enriched housed pigs (Geverink et al., 1999). Geverink et al. (1999) suggested that it may have been possible that in enriched

housed pigs the maximum stimulation of the adrenal cortex was reached at the end of the transport period, which may explain the higher increase in salivary cortisol levels in barren housed pigs as compared to enriched housed pigs. However, Cook et al. (1996) showed that pigs housed in pens with strawbedding may reach salivary cortisol levels of 25 ng/ml after ACTH stimulation, which suggests that maximal stimulation of the adrenal cortex was not reached in E pigs. In addition, differences in concentration of Cortisol Binding Globulin between E and B pigs may contribute to the differences in salivary cortisol responses between E and B pigs. It has been shown that the concentration of Cortisol Binding Globulin in pigs is dependent on a number of variables, e.g. prior exposure to stressors (Cook et al., 1996).

Manipulative behaviour may be stressful for the pigs being manipulated. The significant increase in salivary cortisol levels after transport in B pigs may therefore be due to increased activity and manipulation of pen mates at some time points in the lorry in B pigs as compared to E pigs, that did not show increased cortisol levels after transport. The significant increase in salivary cortisol levels during the lairage period in B pigs may be due to the higher occurrence of fighting at the end of the lairage period in B pigs as compared to E pigs, that also did not show an increase in salivary cortisol levels during the lairage period. Geverink et al. (1996) showed that the rise in cortisol after mixing of pigs is positively correlated to the amount of aggressive behaviour during mixing.

E and B pigs did not differ in latency to leave the home pen and latency to leave the transport box, in contrast to previous experiments showing that B pigs were easier to drive and load than E pigs (Beattie et al., 1995b; Geverink et al., 1999). In contrast, Warriss et al. (1983) reported that extensively housed pigs were more willing to move than intensively housed pigs. The differences between these studies may have been caused by different treatments during the rearing period. The ease of loading may be strongly dependent on treatment of the pigs during rearing, like handling (Grandin, 1997; Hemsworth and Barnett, 1991, 1992) or allowing pigs to leave the pen and explore the passageway during rearing (Geverink et al., 1998). Both E and B pigs in the present experiment were handled and subjected to a maze test with positive reinforcement during rearing (De Jong et al., 2000). This may have caused both E and B pigs to be more willing to leave the pen.

When enriched and barren housed pigs were transported without mixing, no differences in behaviour during transport and in lairage were found between barren and enriched housed pigs (Geverink et al., 1999). However, we showed that after mixing of pigs before transport, which is a common pre-slaughter procedure (Bradshaw et al., 1996), enriched and barren housed pigs differed in behaviour in the lorry and in lairage.

In the lorry, B pigs tended to spend more time on manipulating other pigs, i.e. massaging, nosing and chewing, which may have been stressful for the pigs being manipulated. An increased occurrence of manipulative behaviour in the home pen in barren housed pigs as compared to enriched housed pigs has been found before (Beattie et al., 1995a; De Jong et al., 1998; Fraser et al., 1991; Schouten, 1986). It has been suggested that because of the lack of suitable material for exploration, B pigs redirect their explorative behaviour to the pen mates (Beattie et al., 1995a; Fraser et al., 1991; Schouten, 1986). However, the manipulation of pen mates in the lorry may also be an element of agonistic behaviour. This is supported by the observation that B pigs tended to spent more time fighting than E pigs before transport and in lairage. In addition, it has been shown that pigs reared in a barren environment behave more aggressively than enriched reared pigs (De Jonge et al., 1996; O'Connell and Beattie, 1999). Possibly B pigs were not able to perform the whole repertoire of agonistic behaviour due to the motion of the lorry, but could only perform some agonistic behavioural elements like nosing and massaging of other pigs. It has been shown that nosing is a social interaction that preceeds or occurs during agonistic interactions in pigs (Jensen, 1994).

B pigs also were more active in the lorry and in lairage than E pigs. B pigs tended to spend more time walking and standing, and less time lying in the lorry, and they tended to spend more time walking in lairage than E pigs. This may have been caused by more restlessness in the B group due to increased manipulation of other pigs or fighting, occurring at some time points. However, previous research showed that barren housed pigs had a higher behavioural reactivity to novelty than enriched housed pigs, probably caused by less stimulating housing conditions (Beattie et al., 1995b; Stolba and Wood-Gush, 1980), which may also have played a role. More locomotory behaviour (Beattie et al., 1995b) or more exploration (De Jong et al., 1998; Stolba and Wood-Gush, 1980) in response to a novel environment has been observed in barren housed pigs as compared to enriched

housed pigs. However, we did not observe more explorative behaviour in B pigs than in E pigs in the present experiment.

Previous research showed that enriched housed pigs behave less aggressively than barren housed pigs, because environmental enrichment facilitates the development of social skills (De Jonge et al, 1996; O'Connell and Beattie, 1999; Schouten, 1986). We indeed showed that E and B pigs seem to differ in aggressive behaviour after mixing. B pigs tended to spend more time fighting in lairage than E pigs, especially at the end of the lairage period. Although B pigs spent only 4-6% of the time fighting in lairage, fighting bouts were severe with biting involved.

Replicate effects were found for the time spent walking in lairage. Differences in environmental factors between the two days of slaughter, like a slight difference in environmental temperature, may have contributed to the replicate effects.

In conclusion, results of the present experiment demonstrate that rearing conditions may affect physiological and behavioural responses to mixing at transport and being in lairage. Differences in salivary cortisol responses and behavioural responses indicate that pigs reared in a barren environment may experience more stress during preslaughter procedures than enriched reared pigs. However, more research on the effect of dietary fibre on baseline cortisol levels, and on concentrations of Cortisol Binding Globulin in barren and enriched housed pigs is necessary.

Preslaughter procedures like mixing, driving and transport are obviously stressful procedures that have negative effects on pig welfare (Bradshaw et al., 1996; Grandin, 1997; Warriss et al., 1998). The results of the present study indicate that common preslaughter procedures may have more negative consequences for the welfare of pigs reared in a barren environment than for the welfare of pigs reared in an enriched environment. Because previous research showed that enriched housing conditions are benificial for pig welfare during rearing (e.g. Beattie et al. 1995a, 1996; De Jonge et al., 1996), enriched housing conditions should be preferred for growing pigs.

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